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INFLUENZA EPIDEMICS AND THE INFLUENZA VIRUSES*

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LECTURE I

"Influenza virus A" is the name given by general consent (Horsfall *et al.*, 1940) to the virus originally discovered in 1933 by Wilson Smith, Andrewes, and Laidlaw, who demonstrated that garglings from cases of human influenza would produce a transmissible disease in the ferret. It has since been recovered in many different countries all over the world, and it is now known that there is a group of "A" viruses the individual members of which differ in their antigenic structure but share certain characters; a serological relationship exists between these viruses and the virus of swine influenza, discovered by Shope in 1931. Influenza virus B resembles virus A in many of its properties, but is less pathogenic for the ferret and is absolutely distinct in antigenic make-up. The first virus B strains (Lee and T.M.) were recovered almost simultaneously by Francis and Magill in 1940 in the United States, and, although only a few strains have yet been recovered, it is already clear that a group of viruses exists as in the case of virus A, and that the individual members of the group are more or less closely related to each other. No relation apparently exists immunologically between the groups of A and B viruses.

Methods of Study of Human Influenza Virus Infection

The recovery of influenza viruses in the secretions from nose, throat, or lung, which succeeded well in the first few years after the discovery of virus A, has frequently failed in influenza outbreaks since then, and is not applicable to the majority of cases of virus B influenza. Nevertheless, methods based on recovery of the virus are both convincing and essential to the study of the antigenic make-up of the viruses concerned in different outbreaks, and ferret inoculation and observation remain the classical method. Should inoculation fail to induce signs of infection in the ferret, the development of specific antibodies may yet be observed and furnish useful confirmatory evidence of infection in this animal (Horsfall *et al.*, 1940; Stuart-Harris, Glover, and Mills, 1943). Direct inoculation of chick embryos with filtered human garglings, though successful in the U.S. and Australia, has largely failed in this country, possibly owing to the effect on the embryos of wartime conditions of poultry-raising. Indirect methods of demonstration of virus infection depend on the fact that a sharp increase in titre of antibodies occurs during the course of the illness as measured either by antibodies capable of neutralizing the pathogenic effects of the virus or by complement fixation, or by antibodies capable of inhibiting red-cell agglutination by the virus in the test-tube. The latter method of Hirst (1942) is now universally established as the most practicable means of examining large numbers of sera, and has given excellent results even in the type of influenza most difficult to establish by direct methods—namely, influenza B (Stuart-Harris, Glover, and Mills, 1943). The variable level of antibodies before the occurrence of infection, and technical difficulties, make it essential that a simultaneous examination be made of two sera from each patient—one collected during the first few days of fever, and the second on the eighth to fourteenth day in convalescence. Most observers agree that if a fourfold or greater increase in titre of antibodies develops during the course of illness, influenza virus infection of the appropriate type may be presumed.

Influenza Epidemics in Great Britain from 1932 to 1944

The behaviour of influenza viruses in relation to human epidemics in this country has now been studied in varying degrees of detail over a period of 12 years, which included three years with major prevalences of influenza and four years of minor outbreaks. I shall refer to the Registrar-General's figures for notification of deaths from influenza in the 120-odd great towns of England and Wales with a population of over 50,000, partly because no figures are available for the incidence of simple influenza in the community, and partly because these statistics undoubtedly mirror the incidence of outbreaks in the population at large.

Chart I shows the weekly deaths throughout the year for the three seasons of 1933, 1937, and 1943 with major prevalences of influenza, and illustrates the remarkable similarity between them. These were the winters when influenza virus A was readily demonstrable in the throats of the victims by ferret inoculation and when strains of

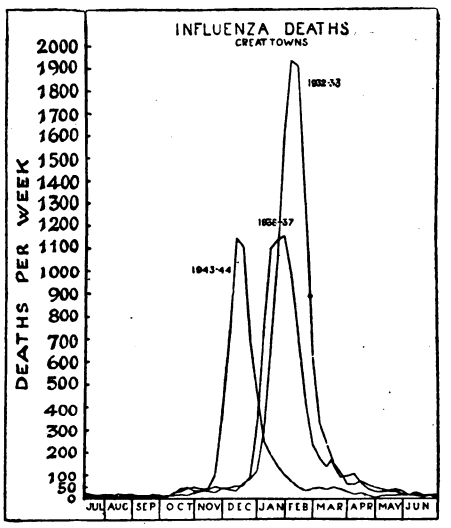

CHART I

virus were subsequently established in the mouse with varying degrees of success. They were the winters when spreading epidemics of influenza occurred in all parts of the country and affected a considerable proportion of the population as well as the semi-isolated communities in which our studies were largely conducted. The British Institute of Public Opinion (1944) estimated that as many

* An abridgment of the Goulstonian Lectures delivered to the Royal College of Physicians on Jan. 16 and 18, 1945.

as 27% of individuals were confined to bed during the period of prevalence of the epidemic in the winter of 1943. Hoyle (1944) estimated, on the basis of complement-fixation studies, that during the same epidemic about 25% of the population were infected with virus A. We do not know the exact time-relationship between the peak of the outbreaks of uncomplicated influenza and the curve for death rates, but it seems probable that the former preceded the latter by two to three weeks in view of the lag between the onset of infection and death and the fact that deaths also occurred from post-influenzal pneumonia. Such an interval was found by Stocks (1944) when he compared the morbidity statistics for Service personnel admitted to E.M.S. hospitals with the curve for deaths for the whole country. The serological tests carried out in the years 1937 and 1943 indicated that at least 80%, and probably more, of the cases of influenza were associated with virus A activity, and no considerable residue of cases unassociated with this virus therefore occurred.

Chart II shows the four years 1934, 1936, 1938, 1942, and early part of 1943, when the notifications gave no indication of influenza outbreaks. In these years either influenza did not break out at all or else local prevalences which were mild and ill defined occurred. A solitary family outbreak in 1934 yielded virus A in the ferret. Several Service outbreaks in the spring of 1936 yielded no evidence of virus A, and were labelled "febrile catarrh" because of the clinical differences from the 1937 virus A cases. No outbreaks were reported in 1938 or in 1942, and in the latter year sporadic cases of clinical influenza in an Army garrison gave no serological changes either to virus A or to virus B. In Jan. and Feb., 1943, months when influenza deaths exceeded 100 in one week, a small increase in sporadic cases of influenza was encountered in two Home

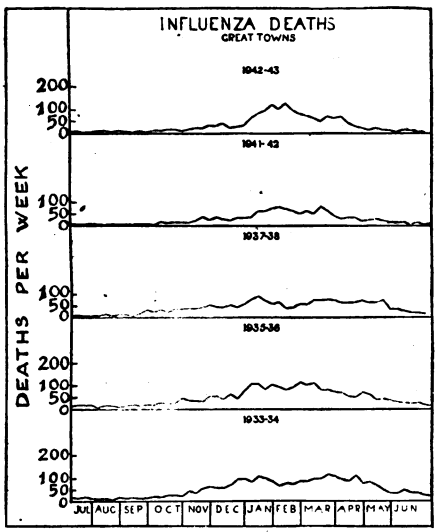

CHART II

Divisions, and one unit experienced a small but explosive outbreak. We found serological evidence of virus B infection in these cases but failed to recover virus (Stansfeld and Stuart-Harris, 1943). The existence of virus B infection was also established serologically in sporadic cases of influenza in London and elsewhere, and Himmelweit established one strain in ferrets from a case of fatal pneumonia (Himmelweit, 1943). However, in this year some 50% of cases, many typical clinically of influenza, yielded no serological response either to virus A or to virus B. Later, in April, 1943, a small outbreak of influenza occurred in an Air Force establishment (Donnelly *et al.*, 1944), and in several scattered outbreaks in summer and autumn. Virus A infection was encountered in tests made at Hampstead (Andrewes and Glover, 1944) on sera from various parts of the country. In this year, therefore, first sporadic cases, then localized outbreaks, and finally a widespread epidemic of influenza A were all encountered.

Chart III shows the figures for the four intermediate years 1935, 1939, 1940, and 1941, when a sustained increase in incidence of deaths occurred between January and March each year yet no sharp peak developed. Localized outbreaks of influenza were recorded in each of these years, but the activity of the viruses was hard to understand. In 1935 two outbreaks in Army garrisons were investigated by Andrewes, Laidlaw, and Smith (1935). In January no virus A was detected in one barracks, yet it was readily recovered in the other in March. Some clinical differences between the cases in the two outbreaks were detected. In 1939 many outbreaks in schools and Service establishments were investigated. A few strains of virus A were recovered, but were difficult to establish in ferrets, and serum tests showed that only 30% of cases were infected with virus A (Stuart-Harris *et al.*, 1940). I could not distinguish clinically between the virus A cases and the others in which infection by this virus was not demonstrable. Following the recovery of virus B by Francis in 1940, the late Dora Lush retested some of our 1939 sera against the virus B strain (Lush *et al.*, 1941). She found several instances of undoubted virus B infection, yet was forced to state that the majority of the sera yielded no better evidence of virus B infection than they had of virus A. In the year 1939, therefore, both virus A and B influenza had occurred.

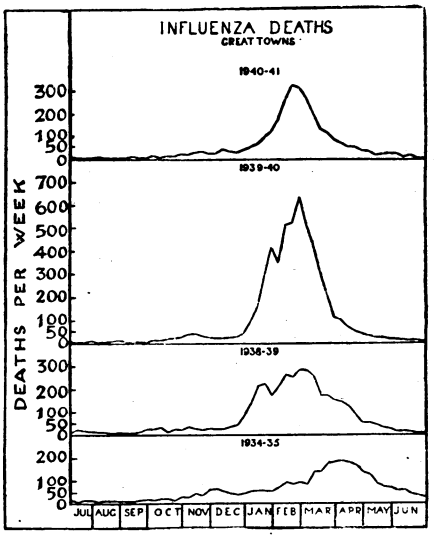

CHART III

but a large proportion of the cases were not demonstrably associated with either virus. Then in 1940, the spring following upon the declaration of war, when mobilization for the Army and evacuation of children had occurred, an unexpected and relatively large wave of influenza-like illnesses was encountered. Owing to the prevailing conditions, attempts to recover viruses directly were the only ones which were employed, and these all proved negative (Andrewes *et al.*, 1941). As no serological tests were made, the outbreaks of this year remain of undetermined aetiology. The last year—that of 1941—will long be remembered by those of us who foolishly imagined that we knew all about the causes of influenza epidemics. It was the winter of the aerial "blitz" on London and the large cities, and it was four years since the previous last widespread epidemic in 1937. When a group of cases in January in Southern England were found to be infected with virus A, and when the virus

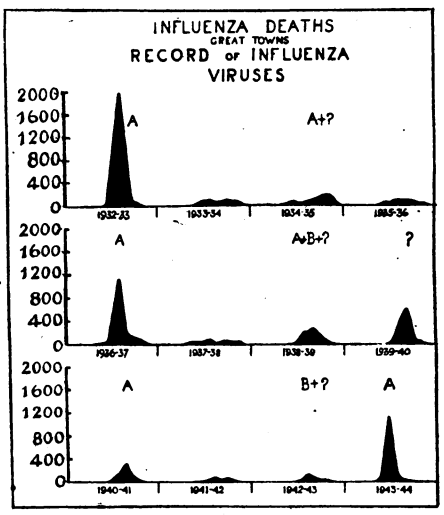

CHART IV

was recovered from garglings from a case of influenza in one of the London shelters, it seemed probable that we were about to witness a serious epidemic. Our gloomy prophecy proved unjustified; no spreading epidemic developed, and we were spared a disaster at this most critical stage of our history. It is remarkable that the chart for death rates showed clearly the small peak coinciding with the recovery of the virus in the laboratory. Nevertheless, the viruses recovered in this year were characterized by their low pathogenicity

for the ferret, and, although no case yielded evidence of virus B infection, at least 20% of the sera tested this year had no evidence of infection either by virus A or by virus B (Andrewes *et al.*, 1941). In all four years, therefore, and in some of the years without peaks in mortality, the minor influenza outbreaks were either A or B, or A plus B, but, in any case, a proportion of cases, varying in number in the different outbreaks, could not be typed. For these cases, which appear to be neither A nor B, the name "influenza Y" has come into use (Rickard *et al.*, 1941). In these years the concatenation of circumstances which is necessary for a spreading epidemic to develop—that is, the state of immunity of the population, the characteristics of the viruses, and possibly other unknown factors—did not occur, and the virus outbreaks remained localized.

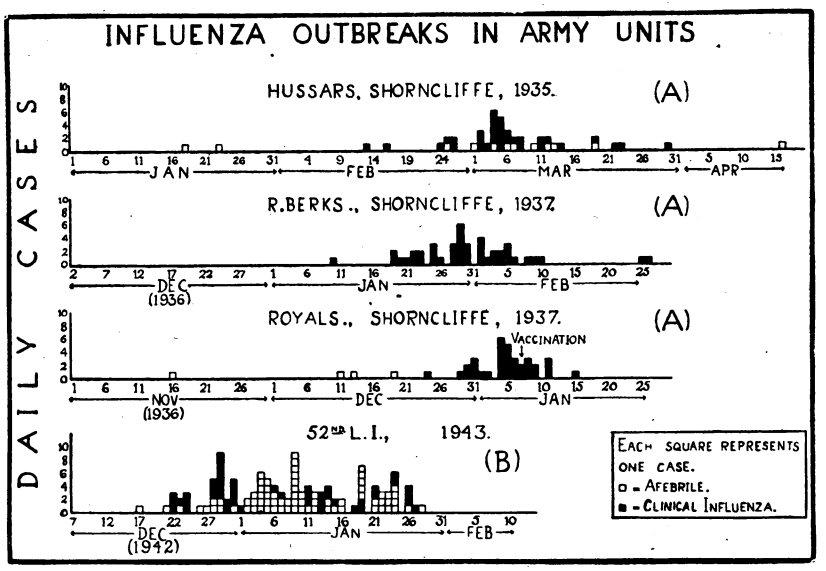

CHART V

Chart IV serves to recapitulate the experience with the viruses in these 12 years and shows in semi-diagrammatic fashion the peaks of mortality from influenza. With the solitary exception of 1943, when cases of virus A influenza occurred in late spring and summer, the records of virus detection in the laboratory which are indicated on the chart represent the only direct evidence of virus activity in these years in Great Britain. No evidence of virus activity or of the presence of virus has been found in between the winter seasons.

Epidemics in Other Countries

Comparison of the experience in Great Britain with that in other countries where studies of influenza virus infection have been made is essential in order to provide us with the proper perspective. Unfortunately, detailed studies have as yet been made in far too few countries for the picture to be anything like complete. Nevertheless, the fact is clear that the majority of widespread epidemics of influenza sweeping through entire countries or continents and causing a rise in the death rate from pneumonia have been associated with virus A infection. Such were the major American and Canadian epidemics of 1937 and 1943, and the smaller prevalences of 1935, 1939, and 1941. These epidemics kept step remarkably with those recorded in Great Britain, and Britain and the North American continent appear to form a closely related epidemiological unit. The virus A outbreaks in Hungary in 1937 and 1939, though coinciding with outbreaks in this country, varied in that much the larger wave occurred in 1939. Also, a widespread epidemic in Russia in 1936, when virus A was identified, occurred at a time when there was no influenza A detectable here. Thus there is some evidence that different parts of Europe experienced their major waves of virus A influenza at different times. Again, the Southern Hemisphere is completely out of step with the Northern Hemisphere. Melbourne experienced major waves of influenza in 1935, 1939, and 1942, and virus A infection was widespread in each of the latter two years. The Argentine had an outbreak of influenza A in 1940, and all these outbreaks in the Southern Hemisphere occurred at the coldest period of the year, between June and September.

Virus B appears to differ from virus A in that it is often not associated with large epidemics. Taylor and others (1942), in recording respiratory infection at a naval establishment in the Argentine, commented on the fact that influenza B occurred in 30% of cases in small waves scattered over several weeks. In Victoria sporadic cases of influenza B occurred in 1943 (Beveridge and Williams, 1944), and in Canada in 1943 was recorded a remarkably similar experience to that in this country, with evidence of 30% case incidence of influenza B in small prevalences in different parts of the Dominion (Hare, Stamatis, and Jackson, 1943). Nevertheless, the initial recovery of virus B was made in the Eastern United States in 1940 at a time when an influenza epidemic of some size was in progress, and outbreaks associated with virus B also affected California in 1936 and 1940 in widespread fashion. While virus B is thus more often than not associated with sporadic cases of influenza, it has at times been associated with large epidemics.

In addition to outbreaks when either virus A or virus B has been detected, various workers have recorded localized mixed outbreaks in which infection by either virus predominated but was accompanied by a variable proportion of sporadic cases due to the other virus. Such were the outbreaks in the United States and West Indies described by Lennette in 1940 and 1941. In these epidemics there were also some cases from which viruses were not recovered and which were unaccompanied by serological change to either virus. These remained in the unclassified group of influenza Y, whose percentage incidence varied widely. Even in the absence of an outbreak, sporadic cases of either influenza A or influenza B have been recorded from time to time. Workers in Australia (Beveridge and Williams, 1943) were particularly struck by the occurrence of sporadic cases of influenza A in 1943, when the incidence of total respiratory infections in various Army camps was too low to be described as epidemic. Sporadic A cases were also encountered in Canada (Hare, Hamilton, and Feasby, 1943) and in the United States (Salk, Menke, and Francis, 1944) in the spring of 1943. Such may merely have been the forerunners of a sequence of events preceding an actual epidemic, as in our own case in the same year, but it is clear that at

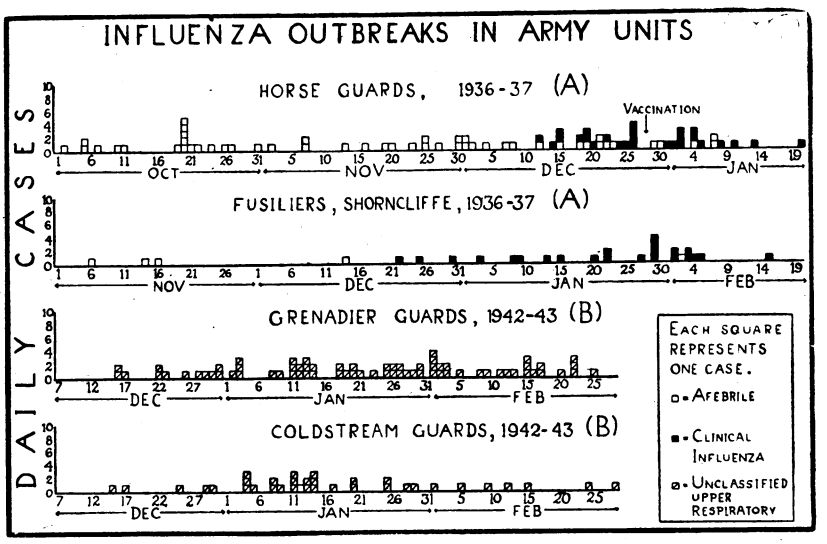

CHART VI

other times sporadic cases have not necessarily presaged an epidemic within a short period of time.

Form of the Influenza Outbreak

When the actual form of an outbreak of influenza in a semi-isolated community is studied, almost every conceivable variation is seen. Chart V shows the sharply peaked type of outbreak seen in regiments not subject to any considerable change of population. The first three of these outbreaks were due to virus A influenza in preponderance and affected 10 to 15% of those at risk, and a proportion of the cases in the fourth outbreak yielded virus B. The large number of afebrile cases in the latter epidemic was noteworthy, and altogether some 13% of the unit were affected. Both viruses have, however, been associated with straggling outbreaks even in the same years as those in which the sharply peaked outbreaks were encountered (Chart VI). The blame for such prolongation of the epidemic wave could not in these cases be laid upon considerable changes in the population at risk, but it is known from other studies that such changes do in fact exert a potent influence on the curve of the epidemic. Thus, in the Services, outbreaks of influenza provoke an unequal incidence of the disease upon the various members of the herd unit, those with lowest total service or shortest duration of residence in the herd being most affected. The accompanying Table shows the

Table showing Incidence of Influenza during Epidemic of 1937 (January to March)

Establishment	Group	No. at Risk	Ages	Influenza (%)	Total Minor Resp. (%)
Chatham Naval Barracks	Recruits	1,440	18–20	12·7	28·2
	Trained men	5,731	18–20	3·8	5·6
Shotley, R.N.	Recruits (boys)	2,005	16	26·7	44·9
	Ship's company	471	—	7·9	10·1
Portsmouth, R.N.	Recruits (boys)	815	16	35·6	53·4
	Ship's company	277	—	6·5	11·9

figures of incidence in Jan.–March, 1937, among men and boys at the Chatham Naval Barracks, when a high proportion of tests in the establishment and elsewhere showed virus A influenza. The recruits with less than 12 months' service suffered five times as heavily as the trained men with over 12 months' service. Similar results were seen at Shotley and Portsmouth Barracks, where trained men furnishing the ship's company had only one-fifth the amount of respiratory infection as had the boy seamen. It is tempting to relate the very high incidence in the boy seamen to the fact that in these establishments monthly intakes of raw recruits occurred and the boys spent only 9 months at each depot. Dudley (1926) has emphasized the paramount importance in droplet infection of the factor of rate of change of population, which is interlinked with the rate of addition of new members to the herd and the duration of residence of each member in the herd. Again, Topley (Greenwood *et al.*, 1936) showed in his experimental epidemics of mouse typhoid and pasteurellosis that without any change in virulence of the organism concerned, waves of renewed epidemic prevalence in the herd could be brought about merely by adding fresh uninfected susceptibles at a steady rate.

It is in the Service training establishments that premonitory waves of the minor respiratory infections, which are always at a higher level than in the population at large, appear sometimes to lead up to actual epidemics of influenza. Possibly this is because the frequent population changes cause frequent interchange of respiratory pathogens and resultant increase in virulence of the agents concerned. Thus at Chatham, in 1936, a definite wave of some respiratory infection not associated with virus A preceded the January wave, when virus A was recovered. Also at the same barracks, in 1939, the January wave, which yielded neither virus A nor virus B, was succeeded after an interval of time by the occurrence of genuine cases of infection with both viruses. A Canadian camp near Toronto experienced three waves in the winter of 1942–3, of which the first was not associated with either A or B, the second consisted of some cases of B, and the third was of virus A influenza (Hare, Hamilton, and Feasby, 1943). Once, however, virus A has again become active, it does not follow that a general epidemic will result immediately, as shown by the recent experience last year. Although virus A was then demonstrated in localized outbreaks in Canada, the U.S.A., and Great Britain in the spring of 1943, and in the last-named country a series of small outbreaks followed during the summer, it was not until the autumn that conditions were ripe for the rapid wave of infection which characterizes a real epidemic of influenza, and this then occurred throughout the population.

This problem of the genesis of an influenza epidemic which is linked with the mode of existence of the virus in between outbreaks has not been solved. There are four possible ways in which the virus could bridge the gap of its existence between epidemics.

First, it might exist by means of a continuous chain of outbreaks, so that always somewhere in the world there is an outbreak or a series of sporadic cases which keeps the virus alive. The general failure to demonstrate the virus in sporadic cases in non-epidemic times must be taken into consideration here. Also, the remarkable way in which over wide areas the viruses may suddenly become active at almost exactly the same time, as happened in 1943, does not suggest that the epidemics in different countries arise by spread of infection from one country to the next. However, the study of isolated communities, as at Spitsbergen or in Greenland, certainly suggests that infection may at times be introduced from outside the community and initiate an epidemic.

Secondly, the virus may live in the nose or throat of healthy carriers in between outbreaks. It has certainly been demonstrated in healthy carriers during an epidemic, and one outbreak of influenza A in an isolated community in Alaska (Pettit, Mudd, and Pepper, 1936) was traced to the arrival of three travellers, themselves unaffected, by 'plane from an area where an influenza epidemic was in progress. Between outbreaks, however, the virus has not been found in healthy persons. Shortly before the war I began to examine tonsils removed at operation from children. In this small series of tests ferrets inoculated with emulsions of the glands failed to show evidence of virus infection. An enormous number of such tests would of course have to be made before negative results could be considered significant.

Thirdly, the virus might transfer its activity to an animal host acting as a reservoir in between human epidemics. It is difficult to know what animal might serve in this capacity, for ordinary laboratory and domestic animals, with the exception of the pig, are not truly susceptible to the virus. In the pig, which has its own particular variety of influenza virus as parasite, the human influenza virus A induces only a very mild disease (Shope and Francis, 1936). The facts that cases of swine influenza in Great Britain have been found to yield strains of virus more closely related to virus A than to the American strain of swine virus (Glover and Andrewes, 1943) and that Shope (1938) found serological evidence of natural infection of pigs with virus A during an outbreak of human influenza may perhaps indicate that under certain conditions the human influenza virus passes from man to the pig.

Fourthly, the virus might exist between epidemics in an unrecognizable form. Such a view was suggested by Andrewes (1942), who postulated the origin of influenza epidemics by the successive mutation of grades of influenza virus. Andrewes thought that sporadic human influenza might be due to a " basic " or rough form of virus devoid of its specific antigen, of animal pathogenicity, and of the power of producing the serological changes due to the normal antigen. This virus would certainly be undetectable by present laboratory methods. Sudden mutation induced by frequent human passages was thought to cause the transformation of basic virus into virus A or B, with characteristic antigen and animal pathogenicity.

Another unrecognizable form of influenza virus has in the analogous disease of swine been clearly demonstrated to be the mode in which the virus persists between outbreaks. Shope (1941) has shown that in swine influenza the virus is already present in masked form in the pig's lung, attached to the lungworm which is such a frequent parasite of the pig. It appears that virus from pigs suffering from swine influenza passes to the exterior via the ova of the lungworm, which are coughed up, swallowed, and pass out in the faeces. The ova develop further into larvae in the earthworm, and when the latter are swallowed by pigs they develop by passing to the lung and are there established. They do not give rise to influenza at once, but by some sort of trigger mechanism, such as changed meteorological conditions, or by artificial means such as repeated intramuscular injection with *Haemophilus influenzae suis*, the dormant virus is activated and the pig develops influenza. An analogous path to the tortuous progress from pig to pig via the lungworm ova and the earthworm could

hardly be conceivable in the case of human influenza, but the general conception of a dormant phase of influenza virus remains as a possible explanation. Epidemics would then arise in communities from virus already seeded but present in a sleeping phase, and would develop because of some changed conditions, perhaps of a meteorological nature, which released the virus from its sleep and caused it to become active once again. These, then, are the various possibilities, and more cannot be said as to which is nearest the truth. It must be admitted, however, not only that the solution of this problem is a matter of academic interest but that it has important practical considerations.

Clinical Manifestations of Influenza Virus Infections

1. Influenza A

As a result of the correlated clinical and laboratory studies made in Britain during the 1937 epidemic (Stuart-Harris *et al.*, 1938) it was thought that the infection produced by virus A could vary from an extremely mild illness through increasing grades of severity to a rapidly fatal pneumonia. It was also considered, as a result of observation of the sera of individuals in contact with cases of influenza but who did not themselves show clinical signs of infection, that subclinical attacks were possible, and probably of frequent occurrence. The results of many studies by different observers all over the world since 1937 have agreed with these views, though little fresh has been added to the clinical data of infection during an epidemic assembled in 1937. Study of the effects of deliberate infection of human volunteers with virus A by Smorodintseff and others (1937), by Burnet and Foley (1940), and by Henle, Henle, and Stokes (1942) has also strengthened the view that the virus is the essential agent in the production of the clinical phenomena observed during an epidemic. The cases which would be recognized by most clinicians as worthy of the diagnosis of influenza thus appear to be merely infections of a particular degree of clinical severity, and are almost certainly outnumbered during an epidemic by subclinical infection and by other cases so mild as to be clinically indistinguishable from endemic colds and the minor maladies of the respiratory tract. Nevertheless, groups of uncomplicated cases of influenza due to virus A do furnish a clinical picture whose recognition from one epidemic to the next has been possible. The picture in the healthy soldier with a typical attack of influenza is as follows:

The patient, usually in good health, is suddenly seized with a headache, and begins to feel shivery and ill. He sleeps poorly, waking at intervals with a cough or with aching in the back or limbs, and in the morning is unfit for duty on account of weakness and fever. He may feel dizzy on rising, may actually vomit or faint. The temperature is usually 100° at this time, and rises steadily to 101° or 102° during the day, symptoms being at their height by the evening. Frontal headache, muscular pains, anorexia, drowsiness, and a desire not to be disturbed are the chief symptoms, but a dry cough, blocked nose, and perhaps sneezing indicate that the respiratory tract is involved. The patient now has a rather characteristic appearance: the face is flushed and slightly bloated, with circumoral pallor and slightly cyanosed lips; the eyes are glistening or slightly injected with excessive secretion and are in part concealed by the eyelids, which droop a little. Apart from fever, and a normal or only slightly elevated pulse rate, nothing much can be made out on physical examination. The tongue is coated with white fur; the nasopharynx is dry, with dilated capillaries; and a rhonchus or group of rales may be heard in the chest, particularly over the lung bases. The next day the temperature has fallen or has begun to fall, and the patient feels much improved. Cough, however, continues, perhaps with pain in the region of the larynx or substernum. The throat may be sore on swallowing and the nasopharyngeal adenoid tissue is swollen. On the third morning there is often a renewal of fever and a return of headache and muscular aching; cough continues, with production of small pellets of mucoid or mucopurulent sputum; and the voice may be a little hoarse The lung bases may show patches of weak breathing and fine rales, but percussion is not impaired, and a skiagram shows no abnormal shadows. Fever subsides after three to five days, and the patient rapidly becomes convalescent, though with cough and slight sputum continuing, and perhaps a tendency to instability of the temperature chart. The leucocyte count remains within normal limits (4 to 14 thousand per c.mm.) throughout the disease, and the relative percentage of the various white cells is not abnormal. Post-influenzal debility and depression are not features of convalescence in soldiers, who are usually fit for duty by the end of the week or the ninth day. Less fit subjects undoubtedly experience these sequelae, and the cough may also take longer to clear up than in fit men. Variations from this typical case, with onset following a premonitory cold, with frequent vomiting on the first day of illness, with more catarrhal symptoms such as acute coryza or a very sore throat, or aphonia, have been encountered, and the temperature chart varies widely. Afebrile cases and very trivial fevers are seen, and many of the 3–5-day fevers have diphasic curves. Prolonged cases of fever usually show persistent physical signs in the chest, and thus grade naturally into cases of influenza complicated by chest involvement. Such cases were divided by Scadding (1937) into three categories, and though his study was based on the civilian population, his findings agreed in the main with our own results on soldiers.

(i) The first group consisted of cases without clinical signs of lung involvement in which there was pharyngitis and tracheitis corresponding to the typical case just described.

(ii) The second group comprised cases with lung signs but without consolidation. These corresponded to cases with bronchitis and bronchiolitis which showed all the usual symptoms of influenza but in addition had areas of diminished movement, perhaps impaired percussion note, weak or suppressed breath sounds, and fine or medium rales scattered in patches usually at the lung bases. In spite of the abundance of physical signs in these patients, skiagrams of the chest showed only vague shadows, which were never as well defined as the clinician expected. It is presumably this type of case which is referred to by some as primary atypical pneumonia due to influenza virus. Apart from the fact that the majority of cases of atypical pneumonia as described in recent outbreaks (Dingle *et al.*, 1944) show *x*-ray abnormalities in excess of clinical expectations, most cases of influenza certainly do not exhibit abnormal *x*-ray appearances. The pathological lesion produced by influenza virus in experimental animals is essentially a necrosis of the epithelium of the finer bronchioles (Straub, 1937), and I would therefore suggest that the majority of the human cases of influenza belonging to this group probably have a bronchiolitis rather than a true involvement of the alveoli.

(iii) Cases with demonstrable consolidation furnished the most severe and also the most baffling of all the variations of the virus infection. The history was that either in continuity with the symptoms of influenza or more usually on the fourth or fifth day, or after a brief interval of a few days from the end of the attack of influenza, pain in the chest and increase in cough and expectoration heralded the development of a lung complication. The course of the pneumonia which followed was recognizably different from that of lobar pneumonia or, for that matter, from other forms of pneumococcal pneumonia. Though this is not the place to enter into detailed descriptions of resemblances and differences, it should be emphasized that the most fulminating cases seen in 1937 were essentially similar to the fulminating pneumonia of 1918 but occurred only rarely, and that a fatal pneumonia was chiefly limited in incidence to the very young and the very old.

The investigations which have thus so far been made on cases of influenza complicated by chest involvement have failed to elucidate the exact relationship of the virus to the pathological changes in the lung. The recovery of virus from cases of influenza with bronchiolitis proved consistent enough in 1937 to indicate that the virus infection coincided with the bronchiolar involvement. In cases of pneumonia, however, the virus was rarely recovered from garglings or sputum by the time that lung signs had developed, though it was recovered, in common with *Staphylococcus aureus*, from the lungs of three rapidly fatal cases (Stuart-Harris *et al.*, 1938). Also, some of the cases of pneumonia already had a high level of neutralizing antibodies in the serum by the time that pneumonia existed, which seemed to indicate that the virus infection often preceded the lung involvement. Hitherto, in this discussion, little attention has been paid to the role of bacteria in the production of disease during an epidemic. In swine influenza the virus alone produces only a mild disease, and simultaneous infection by the virus and the pig variety of *Haemophilus influenzae* is necessary for the full reproduction of the natural disease, which includes consolidation of the lungs. In human influenza the virus alone appears to be fully adequate to produce the uncomplicated disease, but the evidence favours the view that secondary bacterial invasion is necessary for pulmonary involvement, at any rate with the type of human virus known to us in the laboratory. The exact species of bacteria varies in different patients. The pneumococcus is far and away the commonest type to be found either in the pneumonia accompanying or in that which follows virus A influenza. The *Staphylococcus aureus* appears, however, to be particularly important in fulminating types of pneumonia, and a necrosing bronchiolitis and multiple abscess formation are found in such cases at necropsy (Scadding, 1938; Finland *et al.*, 1941). It

may be that the rapidity with which patients with this type of pneumonia die favours recovery of virus from the lung, for fatal cases of pneumococcal pneumonia usually live longer than staphylococcal cases, and virus is not usually recovered from the lungs of pneumococcal cases at necropsy. Disappearance of virus from the lungs of ferrets also occurs at an early stage of infection, and by the sixth day, in spite of the presence of lung lesions, the virus may no longer be detectable by subinoculation to other ferrets. The haemolytic streptococcus has been extremely uncommon as a secondary invader of recent years, and the role of the *Haemophilus influenzae* in pulmonary cases has not been at all clear, although the organism has often been found in the sputum of all types of the disease. Thus it seems clear that the lung consolidations which have been encountered in recent epidemics have not been due to virus alone, although the virus is fully capable of producing lung lesions in the experimental animal and, in any case, may in some way destroy the defence mechanism against bacteria. The possible influence of bacterial infection on that due to the virus, by aiding spread of the virus in the lung, as suggested by Taylor (1941), must also be taken into account. It may be that the current strains of virus, as Burnet and Clark (1942) have suggested, limit their attack to the bronchioles of man, but that only a slight modification of their power of spread down the respiratory tract, with actual involvement of the alveoli, would be needed to enable them to produce a much more lethal type of disease. Meanwhile the remarkable way in which the curve of deaths from pneumonia follows the outbreak of the simple virus disease in the population must indicate an extremely close relationship between the virus and the pathological conditions in the lung, however these may be produced.

2. Influenza B

The only detailed studies of the clinical picture of influenza B which have been made agree in general in finding that there are few differences between the typical attacks of influenza B and those of A. Stansfeld and I in 1943 saw several groups of cases of influenza B and, both by the bedside and by retrospective analysis of symptoms and signs, were unable to differentiate the cases from those of influenza A seen in other years. The majority of the cases were febrile, and the same variation in duration of fever was seen as in influenza A. Cases complicated by lung involvement were not common, and skiagrams of the lungs of cases taken at an American Army laboratory were not abnormal. Other observers have reported a tendency for influenza B to be more insidious in onset than influenza A, often with a premonitory cold (Hare, Hamilton, and Feasby, 1943 ; Beveridge and Williams, 1944) ; and a greater tendency for the symptoms of an acute cold to accompany the fever was noted by Nigg and others (1942) in Minnesota and by Hare, Stamatis, and Jackson in 1943. Some afebrile cases with the same serological changes as febrile ones were encountered by Hare and co-workers in 1943. A number of cases seen by the latter were described as showing the *x*-ray findings of atypical pneumonia, but it seems possible that the high incidence of this syndrome may have been related to the occurrence of numbers of cases of atypical pneumonia in a respiratory wave preceding the occurrence of influenza B. In our own outbreak, cases of influenza B did not show the *x*-ray appearances of atypical pneumonia, and sporadic cases of the latter syndrome occurring at the same time of year as the influenza did not reveal serological changes of influenza virus infection. True consolidation of the lungs was not encountered by us during the prevalence of influenza B, but in the outbreak described by Nigg in 1942 a number of cases of pneumonia from which pneumococci were isolated occurred coincidently with the cases of influenza. Also, Himmelweit in 1943 recorded a fatal case of pneumonia in which the lung contained both virus B and enormous numbers of *Staph. aureus* at necropsy. It does seem probable, however, from the combined experience of influenza B in all parts of the world, that pneumonic complications occur less commonly than in influenza A. Evidence of subclinical infection during an outbreak of influenza B (Nigg *et al.*, 1942) has been obtained as in influenza A, and study of deliberate infection experiments in humans by Francis and others (1944) indicated that subclinical infection can indeed occur among the healthy contacts of individuals infected with the virus.

Differential Diagnosis of Virus Influenza, Sporadic Influenza, and Influenza Y

It has been said that typical attacks of influenza A are recognizable clinically from one year to the next. Yet the variation between the cases is so great that recognition of the disease in the individual is impossible. Furthermore, if groups of cases of sporadic influenza occurring in non-epidemic seasons at all times of the year and not demonstrably associated with either virus A or virus B are compared clinically with groups of virus influenza, sharp distinction cannot be made (Rickard, Lennette, and Horsfall, 1940). Even during actual outbreaks many cases are seen in which the diagnosis of influenza may be made at the bedside, yet no serological evidence of either virus A or virus B infection can be obtained and the viruses cannot be demonstrated in the human secretions. It is this group of cases of clinical influenza referred to already as influenza Y which provides us with a still unsolved problem in aetiology. The percentage of cases of influenza Y varies greatly in different outbreaks, being least in years of large-spreading outbreaks of influenza A and greatest in years of ill-defined outbreaks of influenza B or of mixed A and B infection. Stansfeld (1943) attempted to differentiate the cases without serological change from the neighbouring cases of influenza B, and compared them with the cases of influenza A which I had collected in various years. There were no sharp differences between the three groups, and this experience agrees with our own in 1939, with the findings of Lennette (1941), of Taylor (1942), and of Hare (1943). In 1943 influenza Y comprised some 43% of our cases, but 70% of influenza Y cases was found in outbreaks in Canada (Hare, Stamatis, and Jackson, 1943) and in the Argentine (Taylor *et al.*, 1942).

Although various observers have speculated on the aetiology of influenza Y, it is certain that we do not at present know the answer to this riddle. The various theories may be briefly mentioned. First, there is the obvious theory that these cases are due to a third type of influenza virus so far undiscovered. Secondly, influenza Y may be due to the basic form of influenza virus in its "rough" state without specific antigen, as postulated by Andrewes (1942). Thirdly, there is the theory that these cases are really infections by either virus A or virus B, but that for some reason there has been a failure of serological response, or that our methods are too crude to detect the rise. Evidence for this has been brought forward by Rickard and others (1941), who found that cases of influenza Y possess in their sera in the acute stage of illness a higher content of neutralizing antibody to either virus A or virus B than cases of virus influenza in the same outbreak. It is known that the smallest proportional increase in antibody occurs among individuals with a high level of antibody before infection, and therefore it is conceivable that such antibody increase as does occur in the influenza Y cases is undetectable by our present crude methods. Francis (1944) also encountered cases of infection following deliberate instillation of virus B in which, in spite of a good clinical reaction, serological change was trivial or absent and the virus was not recovered from garglings. Another possibility is that these cases are due to antigenically different strains of the same A or B virus. Magill and Sugg (1944) have pointed out that if a large number of different strains, especially of viruses isolated during an outbreak, are employed in the tests of sera from the same outbreak more cases will be found to show antibody response to at least one strain than if only one strain is used in the test. My personal view would incline me to the belief that another virus, or other viruses, as yet unknown, are the cause of at least some of the influenza Y cases in an outbreak. It would seem logical that if virus B differs from virus A in being much less pathogenic for the ferret, other viruses capable of producing influenza in man exist which are still less pathogenic for the ferret than virus B. The antibody estimations carried out on the cases of influenza Y by us (Stuart-Harris, Glover, and Mills, 1943) showed these cases to occur slightly more often among the groups with high initial antibodies to virus B (Chart VII). Cases did occur with all grades of B antibody, however. The influenza Y cases were also distributed in time throughout the epidemic, though with less incidence in the first two weeks of January, when virus B infection was relatively commoner. Until some method is found of recovering virus from these

individuals, however, the aetiology of influenza Y must remain undecided.

Certain Other Respiratory Diseases Unrelated to Influenza Virus Infection

Francis, in an address in 1937, when discussing the differential diagnosis of influenza said it was easier to describe what influenza was not than to define what it was. This point of view certainly appeals to me, and one seems to be on reasonably certain ground in stating that influenza is not the endemic afebrile cold, is not streptococcal tonsillitis, and is not primary atypical pneumonia. Nor, in recent years at any rate, has it been a gastro-intestinal disease. However, it is certainly true that during an epidemic both influenza A and influenza B can mimic the common cold, and one feels that the common cold virus will eventually be found to be related to the group of influenza viruses and that at times it may cause attacks of clinical influenza. More cannot be said in the absence of knowledge of the antigenic make-up of the common cold virus or until some more reasonable way is found of working with the virus than by studying its effect in the chimpanzee (Dochez *et al.*, 1930). Streptococcal tonsillitis stands more clearly apart from influenza than any of the conditions previously discussed, but there are some cases of exudative tonsillitis in which the haemolytic streptococcus is not concerned that may occur side by side with other cases more clearly resembling clinical influenza. The febrile sore throat or pharyngitis of the medical inspection room does not seem to be influenza, and yet may at times be extremely difficult to distinguish from it. There is a greater tendency for pharyngitis to be accompanied by severe laryngitis and tracheitis, and the name "febrile catarrh," though now discarded, may yet be found to be of use when our knowledge of the virus and bacterial infections of the upper respiratory tract is more complete. The group labelled "primary atypical pneumonia" includes examples of infection by viruses of the psittacosis group (Meiklejohn *et al.*, 1944), but a majority of the cases are of undetermined aetiology.

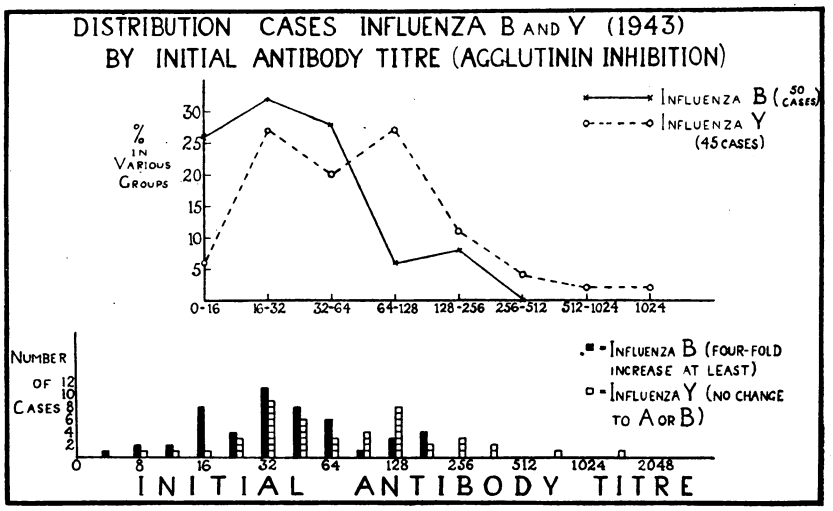

CHART VII

On purely clinical grounds, atypical pneumonia is readily confused with influenza in view of the occurrence of fever, constitutional symptoms, and signs of respiratory-tract infection in both conditions. The onset is more often insidious, the fever lasts longer on the average, and the cough is more often paroxysmal, irritating, and accompanied by substernal discomfort in the case of atypical pneumonia. The characteristic veiled shadows spreading out from the hilar regions which are seen in skiagrams of the chest in atypical pneumonia at once distinguish the condition from simple influenza. Also, the fact that there is more to see in the *x*-ray picture than one would expect from examination of the chest should help to differentiate atypical pneumonia from influenza with bronchiolitis. The discovery of the rise in titre of cold agglutinins for human red cells (Peterson, Ham, and Finland, 1943) which occurs in the serum of many cases of atypical pneumonia during convalescence may be of use when the serological tests for influenza cannot be applied. Further clarification and definition of the atypical pneumonia syndrome from influenza must, however, await, as in the common cold, exact knowledge of the aetiology of the condition. The studies of Eaton *et al.* (1944) on the pulmonary disease in cotton-rats and hamsters induced by sputum from cases of atypical pneumonia deserve special mention in this connexion. In the meantime, the careful studies in progress in the United States (Commission on Acute Respiratory Diseases, U.S. Army, 1944) have led to a recognition of the commonness of the disease, its high incidence in Army recruits, and its almost constant ratio of 10% of the total undifferentiated minor maladies of the respiratory tract in Service personnel.

The Possible Relation between Virus Influenza and Pandemic Influenza

I cannot conclude without a few remarks on the possible relationship between influenza epidemics such as those which I have described and the type of influenza responsible for the 1918 pandemic. Dwight O'Hara (1944) has recently suggested that we owe our present freedom from devastating influenza epidemics to the annual or biennial recurrence of fair-sized outbreaks. A mere glance at the incidence of influenza since the middle of last century is enough to give a bold negative to this theory. The freedom from influenza which prevailed for nearly 50 years after 1847 was, it is true, followed by the pandemic of 1890. But ever after this we have experienced a much greater prevalence of the disease than before 1890, and experience in distant parts of the world, such as Australia, has been much the same. The wartime years which preceded the 1918 wave, far from being years of low prevalence of influenza, were actually periods in which pneumonia mortality and respiratory infections in general increased steadily each year. In the present war, and in spite of unprecedented overcrowding and movement of population within this country, the epidemics have been, if anything, milder than in pre-war years, and there is no suggestion that we are likely to experience the return of a pandemic such as that of 1918. Abler workers than I have speculated upon the relationship between influenza viruses and pandemic influenza, and Burnet and Clark (1942) have given an admirable survey of the possibilities. Analogy between virus A influenza and the pandemic variety breaks down even if only the mild summer wave is considered. For though the three-day fever of June, 1918, was very similar clinically to virus A influenza, the occurrence of a much higher mortality in the young adult of 20 to 30 even in the first wave in 1918 indicates some fundamental difference. The later waves in 1918 not only showed the same trend in age mortality as did the first wave but suffered an incidence of pneumonia of a size entirely unlike present-day experience.

Three main explanations have been advanced to account for the different characteristics of the 1918 pandemic. First, it may have been due to a virus of entirely novel antigenic type which, meeting a wartime and susceptible population, behaved as, for instance, measles behaves in a virgin population not previously exposed to it (Fiji, Faröe, and St. Helena epidemics). The suggestion made by Laidlaw and supported by Shope was that the swine influenza virus now prevalent in the pigs of the Middle West of the United States represents the survival in an animal host of the pandemic human virus (Shope, 1943). Evidence for this is simply that swine influenza first occurred in the United States in 1918, and Koen suggested at that time that it was acquired from human cases. The occurrence of antibodies to swine virus in the sera of adult humans but not of children was at one time thought to favour this view, but it is now known that such antibodies develop in response to infection by human virus A influenza. Secondly, pandemic influenza may have been due to a peculiarly high rate of secon-

dary bacterial invasion. Evidence both for and against this can be assembled, but perhaps the weight of argument is against this theory, because no one type of bacterial species occurred more frequently than any other. The third view is that the virus of 1918 influenza was of the same antigenic character as, for instance, virus A, but possessed unique biological properties (Burnet and Clark, 1942). Andrewes (1942) conceived of pandemic virus as being merely a more advanced grade from the virus of present experience with the added property of attacking the lung, but felt that antigenic mutation would alone explain the lethal effect of the viruses on the young adult. Burnet and Clark explain the latter phenomenon on the basis of excessive reaction of the inflammatory response to a strain of virus capable of spreading down into the alveoli.

Comparison of the properties of the various influenza A viruses encountered in the epidemics of different years certainly supports the view that virus strains of almost the same antigenic constitution can vary considerably in their virulence for experimental animals. It is a striking fact that whenever a considerable epidemic is under way no one seems to find it difficult to establish strains of virus A in the ferret, the clinical responses in the ferret are clear-cut, and passage is successful in a high proportion of instances. The viruses obtained from localized outbreaks of years such as 1939 and 1941 were by contrast difficult to transmit to ferrets, caused poor clinical reactions, and tended to die out on passage unless "nursed" along. We also know something of the variations which can be induced in the virus by simple transfer from one animal to another, so that the virus appears to be biologically plastic and capable of very considerable modification. The studies of Burnet and Bull (1943) on the changes which occur in the influenza virus immediately after isolation from human cases indicate the relative instability of the human virus and the considerable ease with which mutation can be induced. Should a strain of virus arise in nature, as a result, perhaps, of a particular combination of circumstances, which possessed both heightened power of spread and changed antigenicity, then a pandemic with particular mortality in the younger age groups might result. When all is said, however, our theories must remain unproved in the absence of opportunity of putting them to the test, and everyone must agree that ignorance is a more blessed state than knowledge born only of bitter experience of a new pandemic.

To summarize: I have tried to show how the knowledge of the virus agents active in the causation of influenza has begun to illuminate the problem of one of man's most implacable enemies. Whenever the influenza epidemics of recent years in Great Britain have been intense and widely spread they have been associated with that virus designated influenza A. Intervening epidemics have been associated both with virus A and with virus B, but a varying proportion of cases clinically similar to those associated with these viruses have remained of unknown aetiology. The clinical features of infection by the two known groups of influenza viruses have been described, and an attempt has been made to interpret the role of the virus in the pathogenesis of the human disease. The manner in which knowledge of the influenza viruses may help us to interpret pandemic influenza of the type experienced in 1918 has been briefly described, and emphasis has been placed on the variable biological properties of the different virus strains and on their power of rapid modification.

(A full list of references will be given at the end of Lecture II.)

We have received from the librarian of the U.S. Office of War Information, American Embassy (1, Grosvenor Square, W.) the printed report of a discussion on "Putting the Disabled Veteran back to Work." Those who took part included Dr. C. D. Selby, medical consultant, General Motors Corporation, and Dr. Harley L. Krieger, medical director, Ford Motor Company. The debate was held in the Mellon Institute on the occasion of the eighth annual meeting of the Industrial Hygiene Foundation, and the report is published by that body from 4400, Fifth Avenue, Pittsburgh, Pennsylvania. It forms Bulletin No. 2 of the Foundation's special series of publications.

DRYING PENICILLIN BY SUBLIMATION IN THE UNITED STATES AND CANADA

BY

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Desiccation from the frozen state has now become well known as the result of its very wide and extensive application to the drying of blood plasma. The art itself is old, and dates back to Shackell in 1909. The subsequent history of the development of desiccation from the frozen state during the present century has been recorded in previous publications (Flosdorf and Mudd, 1935; Strumia *et al.*, 1941; Flosdorf *et al.*, 1945), and these include also reference to the work of Shackell (1909), Harris and Shackell (1911), Hammer (1911), Rogers (1914), Swift (1921), Sawyer *et al.* (1929), Elser *et al.* (1935), Reichel, Masucci, McAlpine, and Boyer (unpublished; see Flosdorf and Mudd, 1935), Reichel (1939), Greaves and Adair (1939), and Greaves (1942, 1944).

By 1935, as a result of the development made by these various workers and of our own contributions (Flosdorf and Mudd, 1935), desiccation from the frozen state was available for general production of various biological products. Hence when the war came the art had been developed and was ready for use with blood plasma; of course, under the stimulation of a project like this, the process was soon applied on a grander scale than ever before.

Earlier efforts to evaluate the possible clinical usefulness of penicillin were abandoned because its liability seemed to make it unsuited as a therapeutic agent (Clutterbuck *et al.*, 1932). However, when later research had resulted in demonstration of the effectiveness of penicillin (Chain *et al.*, 1940; Abraham *et al.*, 1941), it was natural that desiccation from the frozen state should be called upon to stabilize it; meanwhile, drying this way had undergone the necessary development. In many respects the problems with plasma and penicillin were similar and parts of equipment even identical. On the other hand, certain peculiarities in the nature of penicillin introduced problems quite different from any encountered earlier.

Actually, solutions of penicillin are not so labile that they cannot be evaporated at about room temperature without loss in potency, but they foam badly. Desiccation from the frozen state avoided this problem. This also allowed a more highly soluble product to be obtained.

On the other hand, if penicillin is to be dried while frozen, it is necessary to hold the temperature lower even than that which is required for many other biological products, including blood plasma. This is not because of the biological lability, but because of physical characteristics. Penicillin does not remain in a completely unsoftened condition unless the temperature is below about −20° C., and preferably below −25° C. The exact temperature varies somewhat with the degree of purification and with the concentration of the product; nevertheless, even the least exacting of preparations require a lower temperature than plasma. If penicillin is not kept at such low temperature, because of softening there is bubbling and frothing to a partial extent, and this is sufficient to introduce problems of practical control which are difficult to overcome. Consequently, it is simpler to maintain a lower temperature.

Drying in Bulk or in Ampoules and Bottles

Drying of a biological product intended for parenteral injection may be carried out either in bulk or in the final container in which the product is to be stored and distributed. However, drying in bulk has not met with general favour because of the obvious problems of maintenance of sterility in subdividing dry powder into ampoules for distribution. The problem is made more difficult because of the hygroscopic nature of dry penicillin, the size of doses requiring accurate weighing of small quantities. In spite of this, attempts were made to dry penicillin in bulk for transfer of dried powder to final ampoules, and these represent the first time this has ever been tried in routine large *production* of a biological product. To-day, however, the trend has returned to one of drying in